GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: Dr. Bruce Beutler Address: 1055 North Torrey Pines Rd, Mailstop: SP-293 Contact: Dr. Bruce Beutler

Telephone: 858-784-8610 email: bruce@scripps.edu

DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

Protocol: **NAME OF PCR: MMRRC Strain #31078-UCD,** C57BL/6-*Tlr9*^{M5Btlr} (CpG6)

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): lab uses JumpStart®REDTaq®ReadyMix® (P1107-Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs)

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [x]	94	2	**1**
2. Denaturation	94	0.50	30
3. Annealing } steps 2-3-4 will cycle in sequence	56	0.50	30
4. Elongation	72	1	30
5. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: CpG6 (F)	CAGTTCTAGACGTGAGAAGCAACCC
2: : CpG6 (R)	GGCAGAGAATGAACTCCAGTCCTG
3: CpG6 _seq (F)	GGAGCCGCAAGACTCTATTTG
4: CpG6 _seq (R)	TCACTCTCCTGAAAGATGCATGG

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.076	
(i.e. 3&4)		
(i.e. 1&2&3)		

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES

CpG6 genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.

Primers for PCR amplification

CpG6(F): 5'- CAGTTCTAGACGTGAGAAGCAACCC -3'

CpG6 (R): 5'- GGCAGAGAATGAACTCCAGTCCTG -3'

PCR program

1) 94°C	2:00
2) 94°C	0:15
3) 56°C	0:30
4) 72°C	1:00

5) repeat steps (2-4) 35X

6) 72°C	10:00
7) 4°C	∞

Primers for sequencing

CpG6_seq(F): 5'- GGAGCCGCAAGACTCTATTTG -3'

CpG6_seq(R): 5'- TCACTCTCCTGAAAGATGCATGG -3'

The following sequence of 1076 nucleotides (from Genbank genomic region **NC_000075** for linear DNA sequence of *Tlr9*) is amplified:

```
3175                                                          cagttc

3181 tagacgtgag aagcaaccct ctgcactgtg cctgtggggc agccttcgta gacttactgt

3241 tggaggtgca gaccaaggtg cctggcctgg ctaatggtgt gaagtgtggc agccccggcc

3301 agctgcaggg ccgtagcatc ttcgcgcagg acctgcggct gtgcctggat gaggtcctct

3361 cttgggactg ctttggcctt tcactcttgg ctgtggccgt gggcatggtg gtgcctatac

3421 tgcaccatct ctgcggctgg gacgtctggt actgttttca tctgtgcctg gcatggctac
```

3481 ctttgctagc ccgcagccga cgcagcgccc aaactctccc ttatgatgcc ttcgtggtgt

3541 tcgataaggc acagagcgca gttgccgact gggtgtataa cgagctgcgg gtgcggctgg

3601 aggagcggcg cggccgccga gccctacgct tgtgtctgga ggaccgagat tggctgcctg

3661 gccagacgct cttcgagaac ctctgggctt ccatctatgg gagccgcaag actctatttg

3721 tgctggccca cacggaccgc gtcagtggcc tcctgcgcac cagcttcctg ctggctcagc

3781 agcgcctgtt ggaagaccgc aaggacgtgg tggtgttggt gatcctgcgt ccggatgccc

3841 accgctcccg ctatgtgcga ctgcgccagc gtctctgccg ccagagtgtg ctcttctggc

3901 cccagcagcc caacgggcag gggggcttct gggcccagct gagtacagcc ctgactaggg

3961 acaaccgcca cttctataac cagaacttct gccgg**g**gacc tacagcagaa tagctcagag

4021 caacagctgg aaacagctgc atcttcatgt ctggttcccg agttgctctg cctgccttgc

4081 tctgtcttac tacaccgcta tttggcaagt gcgcaatata tgctaccaag ccaccaggcc

4141 cacggagcaa aggttggctg taaagggtag ttttcttcc atgcatcttt caggagagtg

4201 aagatagaca ccaaacccac acagaacagg actggagttc attctctgcc

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated G is shown in red text.